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Rheological Properties of Enzymatically Isolated Tomato Fruit Cuticle[1]

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Rheological properties were determined for cuticular membranes (CMs) enzymatically isolated from mature tomato (*Lycopersicon esculentum* Mill. cv Pik Red) fruit. The cuticle responded as a viscoelastic polymer in stress-strain studies. Both CM and dewaxed CM expanded and became more elastic and susceptible to fracture when hydrated, suggesting that water plasticized the cuticle. Dewaxing of the CM caused similar changes in elasticity and fracturing, indicating that wax may serve as a supporting filler in the cutin matrix. Exposure of the cuticle to the surfactant Triton X-100 did not significantly affect its rheological properties.

The cuticle is a thin polymeric membrane that covers the aerial parts of terrestrial plants (Cutler et al., 1982) and is the primary barrier to the movement of substances between the plant and its environment (Bukovac et al., 1981). Recent studies established that some nonionic octylphenoxy polyethoxylate surfactants, which are often used to improve the performance of foliar applied chemicals, directly increase cuticular permeability of 1-naphthaleneacetic acid (Knoche and Bukovac, 1993), N^6-BA (Petracek et al., 1993), and 2,4-D (Schönherr, 1993). Although the mechanisms for this enhancement are not clear, this increase may be due to surfactant-induced changes in the cuticular polymer. This hypothesis is supported by an atypical increase in cuticular sorption of TX-100 (Shafer and Bukovac, 1987) near the surfactant concentrations that enhance penetration.

Rheology, as characterized by the response of the polymer to mechanical stress, may provide insight into the mechanisms of cuticular penetration. Properties that affect polymer permeability such as alteration of polymer-chain-segment mobility by inclusion of plasticizers also affect polymer rheology (Rogers and Sternberg, 1971). In this study we examined the effects of hydration and the presence of wax and surfactant on the rheological characteristics of enzymatically isolated cuticles.

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MATERIALS AND METHODS

Plant Material

Tomatoes (*Lycopersicon esculentum* Mill. cv Pik Red) were locally field grown free of applied pesticides. Sections of mature fruit, free of visible defects, were excised, and discs (15 mm in diameter) were prepared with a cork borer. Enzymatic isolation of cuticles was based on the technique of Orgell (1955) as modified by Yamada et al. (1964). The excised discs were incubated in a mixture of cellulase (0.2% [w/v], Sigma), pectinase (4% [w/v], ICN), and NaN_3 (1 mM to prevent fungal and bacterial growth) in sodium citrate buffer (50 mM, pH 4.0). The enzyme solution was changed several times during a 2-week period, after which the isolated cuticles were repeatedly rinsed with distilled water, air dried, and stored at room temperature. DCMs were prepared by batch extracting the soluble cuticular lipids with 10 changes of chloroform:methanol (1:1, v/v) at 50°C over 4 d. Cuticles used for scanning EM, specific weight, and wax content studies were air dried and stored in a desiccator.

Chemicals

Citrate Buffer Solution

Buffer solution for rheology and surfactant sorption studies consisted of 20 mM sodium citrate (pH 3.2) with 1 mM NaN_3 included to inhibit bacterial and fungal growth.

Surfactant

TX-100 (α-[4-(1,1,3,3-tetramethylbutyl)phenyl]-ω-hydroxypoly(oxy-1,2-ethanediyl), Rohm and Haas [Philadelphia, PA]) is a surfactant based on octylphenol condensed with ethylene oxide. The number of ethylene oxide groups per molecule averages 9.5 and follows a Poisson distribution.

Cuticular Properties

Specific weight was determined by weighing 50 CM discs (15 mm in diameter) dried to constant weight in a desiccator. CMs were dewaxed, desiccated to a constant weight, and reweighed. Wax content was determined by the difference between CM and DCM weights. Specific weight was expressed as weight per planar area.

Abbreviations: CM, cuticular membrane; DCM, dewaxed cuticular membrane; N, newton; TX-100, Triton X-100.

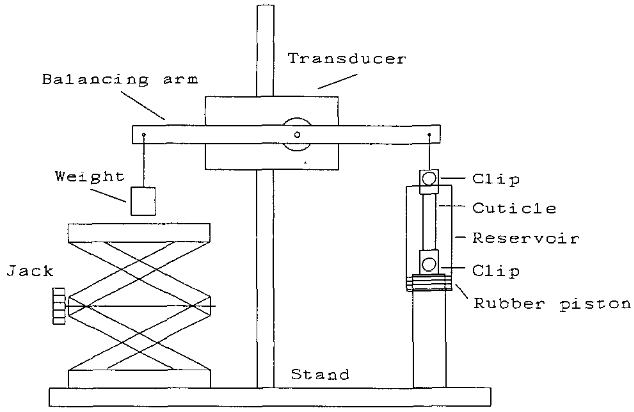

Figure 1. Diagram of the extensiometer equipped with a radial displacement transducer (from Kutschera and Schopfer, 1986).

Density was measured by helium gas pycnometry for CM sections (0.7-g sample) at 25°C by Porous Materials, Inc. (Ithaca, NY). After the initial determination, the CMs were dewaxed, and density determination was repeated. Density values represent the true volume or volume of cuticle not accessible to gas.

Cuticular water content capacity was determined by weighing dry, isolated cuticles and equilibrating (24 h) with buffer solution or buffer solution containing 1.0% (w/v) TX-100. Cuticles were removed from solution, blotted with filter paper to remove free-standing water, and reweighed.

Scanning EM

Freeze-fractured edges of the cuticle were prepared by fracturing isolated cuticles in liquid N_2. Cuticles were mounted on aluminum stubs, gold coated, and observed with a scanning electron microscope (JEOL JSM-35C SEM)

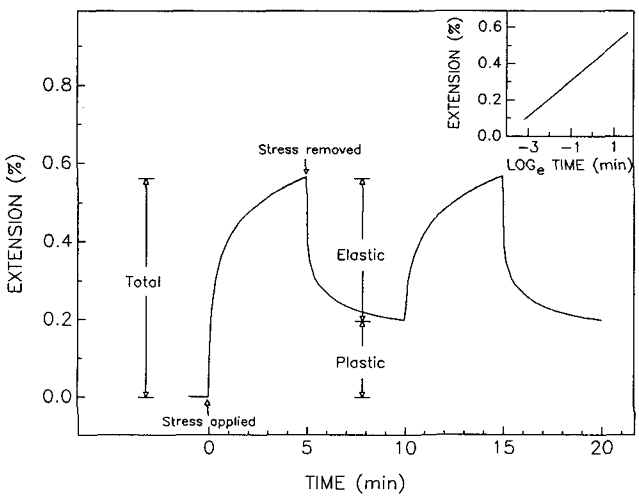

Figure 2. A recorder trace illustrating the change in extension of a dry, enzymatically isolated CM subjected to a force of 0.049 N (5 g weight). After 5 min the weight was removed and applied again after 5 min. Total extension (elastic plus plastic) is a linear-logarithmic function of time (inset).

at 15 kV. Cross-sectional areas for stress-strain calculations were estimated by weighing traces of cuticle cross-sections made from the scanning electron microscope monitor onto paper and converting weight to area. Five samples were examined for each of the five replicates used in the rheology experiments. For stress-strain calculations, only the cross-sectional area of cuticle between the periclinal wall of the epidermal cell and the outer morphological surface was used to express stress (force/area).

Rheological Properties

The rheological properties of the cuticle were measured using an extensiometer equipped with a radial displacement transducer (Kutschera and Schopfer, 1986). Dry cuticle strips (4 × 12 mm) were mounted between clips affixed to a piston attached to a stand and a wire on the balancing arm such that the cuticle formed a plane surface (Fig. 1). Unintentional stretching of the cuticle was minimized by handling the cuticle carefully during mounting and positioning the balancing arm such that only minimal tension was placed on the cuticle during nonstretching periods. After the system was allowed to equilibrate, a reservoir was raised into position around the cuticle. The reservoir was either left empty or filled with buffer solution or buffer solution with 1.0% (15.9 mmol kg^{-1}) TX-100. After re-equilibration (48 h), a transient load creep test was performed. For these experiments, a designated mass was loaded on the balancing arm for 5 min and then removed. The system was allowed to re-equilibrate and the process was repeated for the next mass in the series (3, 5, 8, 10, 13, 15, 18, 20 g ...) until the cuticle fractured. Extension due to transient load as well as hydration (expansion after addition of buffer solution) was followed with a strip-chart recorder. Total extension was expressed as the sum of reversible (elastic) and irreversible (plastic) extension (Fig. 2).

The effect of dewaxing was further examined to determine whether high temperature during solvent extraction affected cuticular rheology. For this experiment, cuticles were (a) held at the temperature used for wax extraction (50°C) for 4 d or (b) repeatedly extracted with solvent (1:1 [v/v] chloroform:methanol) at 22°C.

The data represent average values for cuticles excised from five cuticular segments with each treatment receiving one randomly selected strip from each segment. A factorial

Table I. *Water content and initial linear extension of tomato fruit CM and DCM equilibrated in TX-100*

TX-100 solution consisted of 15.9 mmol kg^{-1} surfactant in buffer. Data for initial linear extension are the means ± SE of five replicates.

Cuticle	Treatment	Water Content	Initial Linear Extension
		g water/g dry cuticle	%
CM	Buffer	0.47	2.6 ± 0.4
CM	TX-100	0.53	3.1 ± 0.3
DCM	Buffer	0.52	2.4 ± 0.3
DCM	TX-100	0.56	3.6 ± 0.5

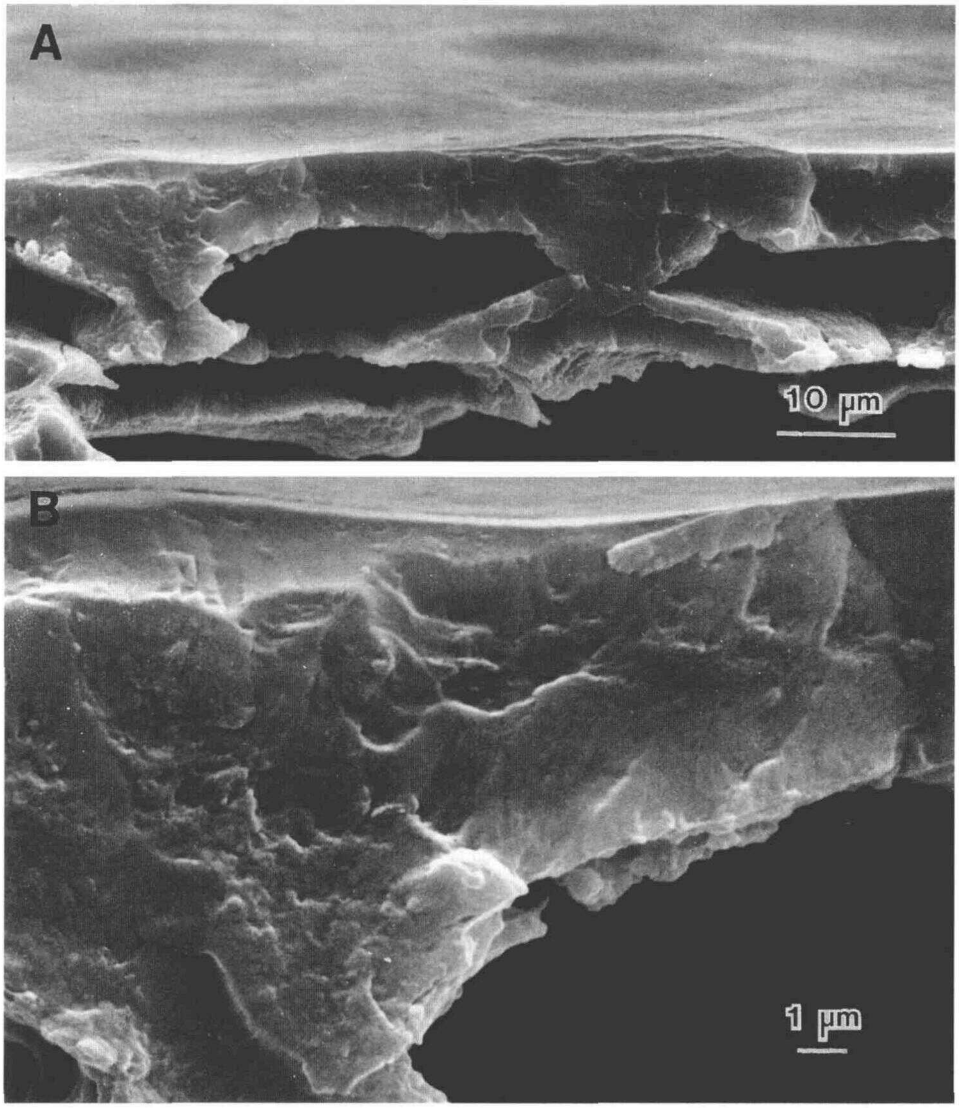

Figure 3. Scanning electron micrographs of a freeze-fractured cross-section of an enzymatically isolated tomato fruit CM. B is an enlargement of an area in A.

analysis was performed on the data using analysis of variance. Data are presented as the means and SE of the five replications.

RESULTS AND DISCUSSION

The tomato fruit CMs used in this study had specific weights of 21.2 and 20.1 g m^{-2} for CM and DCM, respectively. The wax content was 5.2%. CM and DCM densities as determined by helium pycnometry were 1.20 and 1.19 g cm^{-3}, respectively. Water content was similar regardless of the presence of TX-100 in solution or waxes in the cuticle (Table I; range 0.47–0.56 g water/g dry cuticle).

The cuticle was extensively developed, often encasing the epidermal cells and extending into the hypodermal tissue of the fruit (Fig. 3). Average thickness of the cuticle between the epidermal cell wall and the outer morphological surface was 7.0 μm (range 4.4–10.7 μm); average overall thickness (including periclinal regions between epidermal cells) was 10.5 μm (range 6.7–15.3 μm). No pores (stomatal or otherwise) were observed under close examination of about 30 sections of cuticle (approximately 5 $\times$ 5 mm each). Broken trichomes were observed occasionally.

Rheological properties of the cuticle were affected by hydration (buffer treatment) and removal of waxes, whereas surfactant treatment produced no significant effect in addition to that of hydration (Fig. 4; Tables I and II). On hydration, (a) the cuticle expanded about 2 to 4% of total initial length (Table I) in about 3 h, (b) elastic extension increased about 6-fold (Table II), whereas plastic extension was not affected, and (c) fracture force was reduced by 50%. However, the total extension before fracturing of the CM was the same (about 3%) for dry and hydrated cuticles (Fig. 4). Augmenting buffer solution with 1.59 (data not shown) and 15.9 mmol kg^{-1} TX-100 (i.e. 0.1 and 1.0% [w/w] TX-100 to buffer solution) had no effect in addition to that of hydration (Table II).

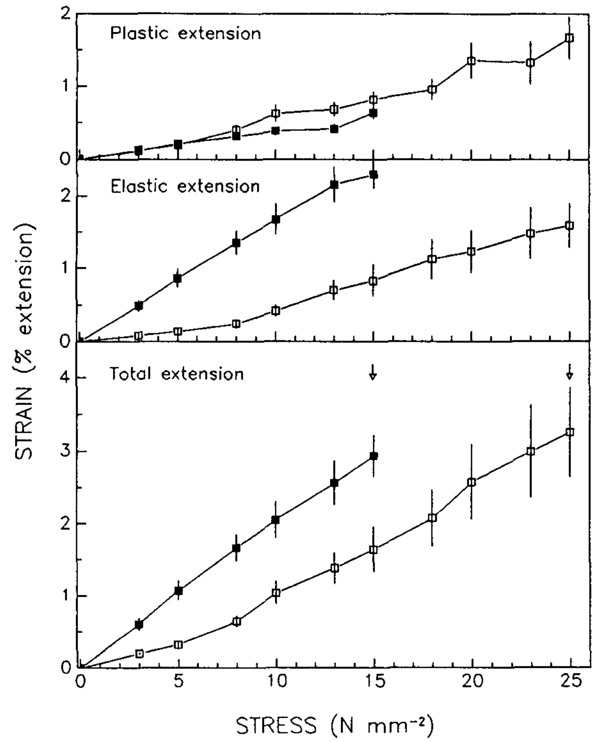

Figure 4. Stress-strain diagrams for hydrated (■) and dry (□) isolated tomato fruit CM. Stress is the force applied per cross-sectional area of the cuticle. Strain is the percentage of extension (total = elastic plus plastic). Arrows represent average fracture force. Bars equal SE for five replicates.

Cuticular compliances (strain/stress or fraction extended per force per area) for a 5-g mass were 0.04 and 0.07 cm^2 N^{-1} for the dry CM and DCM, respectively, and 0.26 and 0.43 cm^2 N^{-1} for hydrated CM and DCM, respectively. In comparison, compliances for synthetic polymers typically range between 0.20 and 2.00 cm^2 N^{-1}. For example, the compliance reported for low-density polyethylene is 0.22 cm^2 N^{-1} (Ferry, 1980).

Hydration and dewaxing produced some similar rheological effects on the cuticle. Removal of waxes nearly doubled elasticity and halved the fracture force (Table II). The effects of hydration and wax removal on elasticity and fracturing were independent of and additive to one another as determined by factorial analysis (P = 0.05). The effect of wax extraction on cuticular rheology was attributed to a solvent effect (1:1 [v/v] chloroform:methanol) and not to temperature (50°C) during wax extraction (data not shown).

Our rheology studies provide three important observations. First, the isolated cuticle may be classified as viscoelastic in nature based on its strain response as functions of time and force (Ferry, 1980). This classification is based on the following: (a) total extensibility comprises both elastic (reversible) and plastic (irreversible) components (Fig. 2), (b) the time response of extension is a linear function of the logarithm of time (Fig. 2, inset), and (c) both plasticity and elasticity are linear functions of stress (Fig. 4). This response to stress with time (Fig. 2) also classifies the cuticle as "leathery," and thus it is above its glassy temperature (Meares, 1965). This state is dependent on cross-linked components for its elastic nature and non-cross-linked components for its plastic nature (Meares, 1965).

Kutschera and Schopfer (1986) used an extensiometer similar to the one used in these experiments and concluded that maize coleoptiles were also viscoelastic. Coleoptile extensibility and factors that affect coleoptile elongation are due primarily to plastic deformation. In contrast, extensibility of the hydrated cuticle is due largely to elastic deformation, and extension is related to factors that affect elasticity.

Second, water affects the mechanical properties of the cuticle by increasing (a) initial linear extension (Table I), (b) elasticity (Table II), and (c) susceptibility to fracture. These features are common for polymers for which solvents plasticize or cause swelling (Meares, 1965; Fels and Li, 1974). Furthermore, water plasticization of the cuticle also may be associated with the reduced rate of sorption of TX-100 by dry cuticles (Petracek, 1991). Tomato fruit cuticles comprise waxes and a polyester cutin matrix (Baker et al., 1982). We assume that the primary sites for water-cuticle interactions are the free hydroxyl groups of the cutin matrix, which may form hydrogen bonds with water. The mechanisms by which water interacts with the cuticle may be important to understanding cuticular penetration of gases and foliar applied chemicals as well as the role of the cuticle in growth and development. These mechanisms must be further examined.

Third, waxes reduce elasticity and susceptibility to fracturing (Table II). These observations support the concept

Table II. *Rheological properties of tomato fruit CM and DCM*

Extension data are the means (± SE) for five replicates for 5 g (0.049 N) of vertical force applied for 5 min at 22°C. Buffer solution consisted of 20 mM citrate (pH 3.2). TX-100 solution consisted of 15.9 mmol kg^{-1} surfactant in buffer solution.

Cuticle	Treatment	Extension: Elastic	Plastic	Total	Fracture Force
			%		*N*
CM	Dry	0.20 ± 0.06	0.29 ± 0.05	0.49 ± 0.05	0.294 ± 0.050
CM	Buffer	1.29 ± 0.17	0.31 ± 0.05	1.60 ± 0.19	0.149 ± 0.015
CM	TX-100	1.36 ± 0.09	0.34 ± 0.03	1.69 ± 0.21	0.145 ± 0.019
DCM	Dry	0.36 ± 0.08	0.44 ± 0.06	0.80 ± 0.07	0.157 ± 0.031
DCM	Buffer	2.16 ± 0.37	0.42 ± 0.09	2.58 ± 0.44	0.092 ± 0.011
DCM	TX-100	1.75 ± 0.11	0.34 ± 0.07	2.09 ± 0.04	0.087 ± 0.020

that waxes act as polymer fillers (Meares, 1965). As fillers, waxes reduce the cuticular matrix mobility, thus effectively mimicking a cross-linking agent that increases rigidity of the flexible cutin matrix (Zlotnik-Mazori and Stark, 1988).

Polymer viscoelasticity and permeability both depend on the rearrangement of the conformation of molecules (Rogers and Sternberg, 1971). Since surfactants increase cuticular permeability (Knoche and Bukovac, 1993; Petracek et al., 1993; Schönherr, 1993) and diffusion is dependent on polymer segment mobility for the creation of free volume (Rogers and Sternberg, 1971), we anticipated surfactant enhancement of cuticular elasticity similar to that caused by hydration (Table II). The lack of any surfactant effect on cuticle rheological properties in this study is surprising, particularly since surfactant constitutes 10% of the total weight of the cuticle when equilibrated with 15.9 mmol kg^{-1} (1.0% [w/v]) TX-100, whereas 30% is water and 60% is cuticle (calculated from Table I and Shafer and Bukovac, 1987). Regardless, either surfactant effects on viscoelasticity are not measurable by the technique used or surfactant effects on permeability are not related to rheological properties. Furthermore, the increased cuticular elasticity and fracturability due to hydration suggest that water may play a direct role in cuticular permeability, fruit peel cracking, and cuticular restriction of cell expansion and thus warrant future study of water-cuticle interactions.

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